Rejections Under 35 U.S.C. § 103

Claims 1-3 and 6 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Trends Biotech 9:226-231, 1991 by Bulow et al. ("Bulow"), in view of J. Biol. Chem. 262:97-102, 1987 by Peoples et al. ("Peoples (1987)") and Mol. Microbiol. 3:349-357, 1989 by Peoples et al. ("Peoples (1989)"). Claim 4 was rejected under 35 U.S.C. § 103(a) as being unpatentable over Trends Biotech 9:226-231, 1991 by Bulow et al. ("Bulow"), in view of J. Biol. Chem. 262:97-102, 1987 by Peoples et al. ("Peoples (1987)") and Mol. Microbiol. 3:349-357, 1989 by Peoples et al. ("Peoples (1989)") as applied to claims 1-3 and 6 above and in further view of J. Mol. Biol. 211: 943-958, 1990 by Argos ("Argos"). Claim 5 was rejected under 35 U.S.C. § 103(a) as being unpatentable over Trends Biotech 9:226-231, 1991 by Bulow et al. ("Bulow"), in view of J. Biol. Chem. 262:97-102, 1987 by Peoples et al. ("Peoples (1987)") and Mol. Microbiol. 3:349-357, 1989 by Peoples et al. ("Peoples (1989)") as applied to claims 1-3 and 6 above and in further view of US Patent 5,610,041 to Somerville ("Somerville"). Applicants respectfully traverse these rejections to the extent that they are applied to the claims as amended.

There is no expectation of success

The examiner has identified prior art that describes the enzymes in issue, and the way they act naturally to produce polymer, as well as references that describe the general desirability of fusion proteins.

What the examiner has not provided is that one of skill in the art would have had a reasonable expectation of success. Indeed, there are several factors that would lead one of skill in the art to be surprised that such fusion proteins could be made and would be active.

(1) As the examiner has noted, the genes encoding these enzymes are present in certain bacteria together in a single locus. However, each gene is under the control of a separate promoter and is separately expressed, not as a fusion protein. If there was an advantage to a fusion protein, or indeed no negative selection away from a fusion protein, one would expect to find mutants in which the stop codon had been omitted and fusion proteins produced. No such mutant has been identified, however.

(2) The proteins are large and their active sites not well characterized. It is well known that the coupling of two enzymes can result in steric hindrance, aggregation, and other problems. See the art cited by the examiner: Bullow and Mosbach, page 227, col. 2.

(3) The genes for these enzymes had been cloned and expressed in bacteria for ten years prior to these fusions being designed, yet no one had made fusion proteins. This is indicative of long felt need.

(4) Unlike most enzymes, these enzymes actually interact not only with substrate but are found bound to the polymer granules. See application page 10, line 16-page 11, line6.

The applicants respectfully submit that the presently claimed invention of fusing multimeric enzyme(s) which require the use of cofactors and which interact to synthesize polymer, has not been previously demonstrated to be expressible as fusion proteins (page 5, lines 22-24). In light of the prior art, there must be a reasonable expectation of success, as determined by one of ordinary skill in the art, of the claimed subject matter (Please see MPEP § 2143.02). Before the results of the present application, one would have reasonably expected the claimed expressed fusions to result in the aggregation of large complexes that are non-functional. The

results provided in the Examples illustrate that this is not the case and Table 1 explicitly indicates that the fusions are functional and result in the production of high levels of PHB. One of skill in the art will realize that this result is not possible if the fusion(s) results in an aggregate of subunits, or even aggregates of the fused proteins themselves, as would be expected before the presently claimed invention.

Bulow

Bulow states that short linkers (two to ten amino acids in length) are optimal and that longer linkers are often prone to proteolytic degradation and the yield of recombinant enzymes can be reduced severely. Bulow further states *for the enzymes described in the article*, correct folding is not impaired and the active sites remain accessible using these short linkers.

Applicant's respectfully submit that Bulow's disclosure of a tryptophan synthase fusion is not predictive of the claimed fusions. Bulow discloses a linker linking different subunits of the *same* enzyme. The present invention is directed to the fusion of two *separate* enzymes, E1 and E2. Bifunctional enzymes, as disclosed by Bulow, are separated into two functional domains and then linked. This is completely different from the presently claimed two enzyme fusion.

The use of fusions in ELISA assays, as disclosed by Bulow, does not permit successive reactions in a pathway leading to the production of polymer. The ELISA fusions in Bulow do allow for enzymatic reactions that are *independent* of one another. This independent nature is completely different from the present invention because the substrate for E2 activity of the present invention is produced by E1. Therefore E2 functionality is dependent upon the activity of E1 and it's product.

Argos

In view of the discussion provided for the Bulow reference above, one would not have been able to incorporate the linkers of Argos in a β-ketothiolase-acetoacetyl CoA reductase fusion with a reasonable expectation of success. Argos admits that "further experiments are required to determine the most important of the various linker characteristics; namely, linker length, *composition*, sequence, *geometry* and the *nature of the genes fused*" (page 956, last column). As discussed above, the nature of the claimed gene products (wherein at least one is a multimer) would prohibit one of skill in the art from reasonably concluding that the linkers disclosed in Argos could be incorporated and be the functional equivalent of those shown in the examples and claimed. Threonine and serine do differ structurally and therefore do not provide the same geometrical configuration within a linker.

Applicant's respectfully submit that Argo's disclosure of immunoglobulin fusions is not predictive of the present invention. Argo discloses a linker linking different domains of the *same* immunoglobulin or enzyme or protein. The present invention is directed to the fusion of two *separate* enzymes, E1 and E2. The separate domains of a *single* protein, as disclosed by Argos, are linked based upon computational modeling. This is completely different from the presently claimed *two* enzyme fusion.

Peoples (1987)

Peoples (1987) describes the cloning, nucleotide and amino acid sequences *of Zoogloea ramigera* β-ketothiolase and expression in *E. coli*.

Peoples (1989)

Peoples (1989) discloses the cloning, nucleotide and amino acid sequences *of Zoogloea ramigera* acetoacetyl CoA reductase and expression in *E. coli*.

Somerville

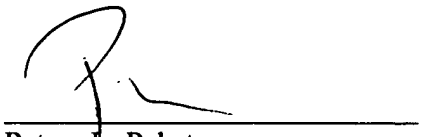
Somerville discloses the expression of separate β-ketothiolase or acetoactyl CoA reductase in *Arabidopsis thaliana*. Somerville does not teach construction or expression of any fusion protein.

Summary

In summary, the applicant's respectfully submit that the teachings of Somerville combined with the teaching of Bulow would not provide one of skill in the art with a reasonable expectation of success of fusing proteins, based upon the foregoing arguments. Previous to Applicants' work, fusing of two proteins, linked by a peptide of between zero and fifty amino acids (Gly and Ser), for successful expression and activity in plants or bacteria had not been accomplished. Based upon the prior art, one of skill would not have reasonably expected the present invention to be successful. This is particularly the case considering the unusual relationship between these enzymes and their reaction production, the polyhydroxyalkanoate granules.

Allowance of claims 1-6 is respectfully solicited.

Respectfully submitted,



Patrea L. Pabst
Reg. No. 31,284

Date: November 1, 2001

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473 (Telephone)
(404) 817-8588 (Telefax)

Certificate of Mailing Under 37 C.F.R. § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231.

Patrea Pabst

Date: November 1, 2001

Marked Up Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Three times amended) A protein fusion having a formula selected from the group consisting of E1-L_n-E2 and E2-L_n-E1, wherein E1 and E2 catalyze successive reactions in a polyhydroxyalkanoate biosynthetic pathway and are each selected from the group consisting of β-ketothiolases, acyl-CoA reductases, polyhydroxyalkanoate synthases, poly(3-hydroxybutyrate) synthases, phasins, enoyl-CoA hydratases, and beta-hydroxyacyl-ACP::coenzyme-A transferase, in which linker L_n is a peptide of n amino acids that link E1 to E2 or E2 to E1, and wherein the fusion protein is under the control of a single promoter resulting in expression of both catalytically active E1 and E2.

2. (Once amended) The fusion of claim 1 wherein E1 and E2 are selected from the group consisting of beta-ketothiolase (phbA) and acyl-CoA reductase (phbB); phbB and phbA; PHA synthase (phaC) and phasin (phaP); phaP and phaC (1D); phaC and beta-hydroxyacyl-ACP::coenzyme-A transferase (phbG); phbG and phaC; phaC and enoyl-CoA hydratases (phaJ); and phaJ and phaC.

3. The fusion of claim 1 wherein n in the linker is between zero and 50 amino acids.

4. The fusion of claim 1 wherein the linker is glycine-serine.

5. The fusion of claim 1 expressed in a plant.

6. The fusion of claim 1 expressed in a bacteria.

Clean Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Three times amended) A protein fusion having a formula selected from the group consisting of E1-L_n-E2 and E2-L_n-E1, wherein E1 and E2 catalyze successive reactions in a polyhydroxyalkanoate biosynthetic pathway and are each selected from the group consisting of β-ketothiolases, acyl-CoA reductases, polyhydroxyalkanoate synthases, poly(3-hydroxybutyrate) synthases, phasins, enoyl-CoA hydratases, and beta-hydroxyacyl-ACP::coenzyme-A transferase, in which linker L_n is a peptide of n amino acids that link E1 to E2 or E2 to E1, and wherein at least one of E1 and E2 is a multimeric enzyme.

2. (Once amended) The fusion of claim 1 wherein E1 and E2 are selected from the group consisting of beta-ketothiolase (phbA) and acyl-CoA reductase (phbB); phbB and phbA; PHA synthase (phaC) and phasin (phaP); phaP and phaC (1D); phaC and beta-hydroxyacyl-ACP::coenzyme-A transferase (phbG); phbG and phaC; phaC and enoyl-CoA hydratases (phaJ); and phaJ and phaC.

3. The fusion of claim 1 wherein n in the linker is between zero and 50 amino acids.

4. The fusion of claim 1 wherein the linker is glycine-serine.

5. The fusion of claim 1 expressed in a plant.

6. The fusion of claim 1 expressed in a bacteria.

U.S.S.N. *
Filed: *
MARKED UP VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

Marked Up Version of Amended Specification Paragraphs
Pursuant to 37 C.F.R. § 1.121(b)(1)(iii)

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